

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

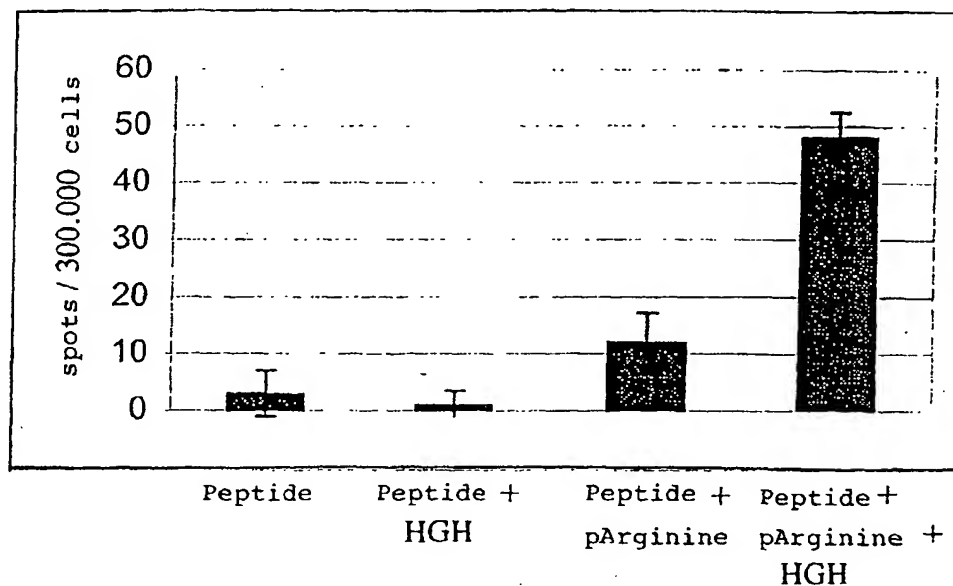
(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number
WO 01/24822 A2

- (51) International Patent Classification⁷: A61K 39/39, 267, A-1190 Vienna (AT). BUSCHLE, Michael [DE/AT]; Hyrtlstrasse 35/1/1, A-2345 Brunn am Gebirge (AT).
A61P 31/00, 35/00, 37/00 MELLING, Jack [GB/GB]; 1 Folly Close, Salisbury, Wiltshire SP2 8BU (GB).
- (21) International Application Number: PCT/EP00/09657
- (22) International Filing Date: 2 October 2000 (02.10.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: A 1680/99 1 October 1999 (01.10.1999) AT
- (71) Applicant (for all designated States except US): CISTEM BIOTECHNOLOGIES GMBH [AT/AT]; Rennweg 95 B, A-1030 Vienna (AT).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): FLEITMANN, Julia-Kristina [DE/AT]; Hauptstrasse 89, A-2463 Gallbrunn (AT). MATTNER, Frank [DE/AT]; Krottenbachstrasse
- (74) Agents: ALGE, Daniel, et al.; Riemergasse 14. A-1010 Vienna (AT).
- (81) Designated States (national): AU, BR, CA, CN, CZ, HU, ID, IN, IS, JP, KR, MX, NO, NZ, PL, SG, SK, US, ZA.
- (84) Designated States (regional): Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
- Published:
— Without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING AN ANTIGEN



(57) Abstract: The invention discloses a pharmaceutical composition comprising an antigen, an immunostimulating substance selected from neuroactive compounds, hormones, compounds having a growth hormone activity, and mixtures thereof, and a polycationic polymer.

WO 01/24822 A2

Pharmaceutical composition comprising an antigen

The invention relates to a pharmaceutical composition especially to be used as a vaccine.

Vaccines are a very successful, yet cost saving medical intervention. Several catastrophic illnesses including small pox and poliomyelitis have been, due to intense vaccination programmes, eliminated from the face of this earth or are on the brink of extinction (Nossal, Nat Med 4, (1998), 475-476). In fact, vaccines can save more lives (and money) than any other medical intervention. Although this notion is valid for a whole panel of diseases including tuberculosis, diphtheria, pertussis, measles and tetanus, there are no effective vaccines for numerous ailments including most viral infections, such as AIDS, and other illnesses including malaria, or even cancer. In addition, the rapid emergence of antibiotic resistant bacteria and microorganisms calls for alternative treatments with vaccines being a logical choice. Finally, the great need for vaccines is also illustrated by the fact that infectious diseases, rather than cardiovascular disorders or cancer or injuries remain the largest cause of death and disability in the world (Bloom et al, Nat Med 4, (1998), 480-484).

The main problem in the field of vaccines is that traditional vaccines (and/or the immune modulating compounds contained within these preparations) are designed to induce high levels of antibodies (Harlow et al, Cold Spring Harbor: Cold Spring Harbor Laboratory, (1988)). Unfortunately, antibodies on their own are not effective in preventing many diseases including most illnesses caused by viruses, intracellular bacteria, or certain parasites. Examples are pathogens such as the above-mentioned HIV virus or Plasmodium spec. in case of malaria. In addition, these vaccines likely will not be effective in cancer. In numerous experimental systems it has been shown that the cellular arm of the immune system, including T cells, rather than the humoral arm, is important for these indications. Therefore, novel, innovative technologies to overcome the limitations of conventional vaccines are needed. The focus must be on technologies that reliably induce the cellular immune system, including antigen specific T

CONFIRMATION COPY

cells, which recognize molecules expressed on pathogen infected cells. Ideally, vaccines are designed that induce both T cells distinguishing diseased, and/or infected cells from normal cells and, simultaneously, antibodies secreted by B cells recognizing pathogens in extracellular compartments.

Commonly, vaccines are administered as a combination of pathogen-derived antigens together with compounds that induce or enhance immune responses against these antigens (these compounds are usually termed adjuvants). Examples of antigens are whole organisms such as inactivated or attenuated viruses or bacteria, fungi, protozoa or even cancer cells. Antigens may also consist of sub-fractions of these organism/tissues, proteins or, in their most simple form, peptides. Antigens can also be recognized by the immune system in form of glycosylated proteins or peptides and may also be or contain polysaccharides or lipids. Short peptides can be used since for example cytotoxic T cells recognize antigens in form of short usually 8-11 amino acids long peptides in conjunction with major histocompatibility complex (MHC) (Rammensee et al., Immunogenetics 41, (1995), 178-228). B cells recognize longer peptides starting at around 15 amino acids (Harlow et al, Cold Spring Harbor: Cold Spring Harbor Laboratory, (1988)). By contrast to T cell epitopes the three dimensional structure of B cell antigens may also be important for recognition by antibodies. In order to obtain sustained, antigen-specific immune responses, adjuvants need to trigger immune cascades that involve all cells of the immune system necessary. Primarily, said adjuvants are acting, but are not restricted in their mode of action, on so-called antigen presenting cells (APCs). These cells usually first encounter the antigen(s) followed by presentation of processed or unmodified antigen to immune effector cells. Intermediate cell types may also be involved. Only effector cells with the appropriate specificity are activated in a productive immune response. The adjuvant may also locally retain antigens and co-injected other factors. In addition the adjuvant may act as a chemoattractant for other immune cells or may act locally and/or systemically as a stimulating agent for the immune system.

Human growth hormone (HGH) is a pituitary-derived factor primarily described for its ability to promote growth acceleration (re-

viewed in Neely et al, *Annu Rev Med* 45, (1994), 407-420). The first patient was treated with growth hormone obtained from pituitary extracts as early as 1958. Recombinant HGH is available for roughly 15 years now and has been used extensively in the clinic. Side effects of recombinant HGH are rare. Efficacy of recombinant HGH preparations has been demonstrated in a wide spectrum of diseases including Turner syndrome, idiopathic short stature, growth hormone deficiency and renal failure.

Whilst numerous studies have confirmed the growth promoting effect of HGH, relatively few reports address a possible interaction of this molecule with cells of the immune system. Stephenson and Melling, who showed that HGH greatly enhances the efficacy of a viral vaccine preparation, first demonstrated the usefulness of HGH in a vaccine context (Stephenson et al., *J Infect Dis* 164, (1991), 188-191). They co-injected HGH with a vaccine for tick-born encephalitis (TBE) virus, an endemic virus transmitted by ticks. In animal experiments HGH potentiated vaccine efficacy and led to the protection of animals after only one injection of the vaccine. The mechanism of how HGH enhanced vaccine efficacy is unclear, but it was speculated that cell mediated immunity played a significant role. There is further, albeit circumstantial, evidence that HGH may indeed induce cellular immune reactions: Mellado et al. demonstrated that if an antigen derived from human immuno deficiency virus (HIV) is applied to mice, they develop a so called Th1 type T helper cell response indicative of a cellular immune response (Mellado et al., *Vaccine* 16, (1998), 1111-1115). Taken together, there is circumstantial evidence that HGH, which is viewed as exemplary for a whole class of primarily neuroactive compounds (see e.g. Levite, *PNAS* 95 (1998), 12544-12549, Scholzen et al, *Exp. Dermatol.* 7 (1998), 81-96), may have a positive effect on the immune system, but the mechanisms remain unclear.

Polycationic polymers, for example the polycationic amino acid polymers poly-L-arginine and poly-L-lysine, have been shown to allow very efficient charging of antigen presenting cells (APCs) with antigens in vitro and in vivo (Buschle et al., *Gene Ther Mol Biol* 1, (1998), 309-321; Buschle et al., *Proc Natl Acad Sci USA* 94, (1997), 3256-3261; Schmidt et al., *Proc Natl Acad Sci USA* 94,

(1997), 3262-3267). This is thought to be the key event for triggering immune cascades eventually leading to the induction of antigen specific immune effector cells that are able to destroy or neutralize targets. It has been shown previously that a number of polycationic compounds exert effects on immune cells (Buschle et al., Gene Ther Mol Biol 1, (1998), 309-321; Buschle et al., Proc Natl Acad Sci USA 94, (1997), 3256-3261;).

Co-injection of a mixture of poly-L-arginine or poly-L-lysine together with an appropriate antigen as a vaccine protect animals from tumor growth in several animal models (Buschle et al., Gene Ther Mol Biol 1, (1998), 309-321; Schmidt et al., Proc Natl Acad Sci USA 94, (1997), 3262-3267). Thus, a vaccine consisting of polycationic compounds and antigen(s) is accepted in the art as being a very effective form of treatment.

GB 1 290 141 discloses a vaccine containing antigenic material with a base-rich peptide as an adjuvant. According to this document the vaccine may consist (as antigenic material) of live or killed organisms, whole or disrupted, or of preparations of natural toxins or products of the organism, or of preparations of extracts of the organisms, alone or in combination with one another. The base-rich peptide used as an adjuvant must contain at least 50% residues which have a free amino group, such as polylysine, polyornithine, polyarginine and polydiaminobutyric acids. In WO 97/30721 the use of basic polyamino acid as preferred adjuvant for vaccines in combination with an immunomodulating peptide or protein (fragment) is described. The use of a further immunostimulating substance to be used together with an antigen and such a polycationic polymer is neither disclosed nor made obvious by these two documents.

US patent 3 725 545 describes that it is possible to potentiate the antibody production ability of nucleic acids containing preparations by adding cationically charged polymers in combination with single-stranded or multi-stranded nucleic acid polymers. Examples of such polycationic polymers are polyornithine, lysozyme, DEAE-Dextran, histone, hexadimethrine bromide and polylysine.

WO 91/04052 also relates to DEAE-Dextran as a polycationic adjuvant in a vaccine composition. This polycationic adjuvant is incorporated into a vaccine which further comprises the antigenic substance (the antigen) and saponin as a further adjuvant. Of course, neither polycationic compounds nor saponin as combined adjuvants may be regarded as an immunostimulating substance within the course of the present invention.

It is the object of the present invention to provide a pharmaceutical composition which allows an effective delivery to a target cell especially to the cellular immune system.

This object is solved by a pharmaceutical composition comprising

- an antigen,
- an immunostimulating substance selected from neuroactive compounds, hormones, compounds having a growth hormone activity and mixtures thereof, and
- a polycationic polymer.

It has surprisingly turned out that the combination of the selected immunostimulating substance according to the present invention and the polycationic polymer with an antigen leads to a synergistic immunomodulating effect for a given antigen preparation. Indeed, it turned out that the effect of an immunostimulating substance selected according to the present invention alone with the antigen has - for itself - even a lower effect than administering the antigen with the polycationic polymer alone.

The immunostimulating effect of substances such as human growth hormone (HGH) has been reported (see also: EP 0 434 749 B1, US 4,837,202, US 5,830,877 and US 5,583,109). Indeed, many of the neuroactive substances, such as pituitary growth hormones also exhibit T cell inducing activity or cytokine secretion altering activities. These compounds also may act on APCs.

The immunostimulating effect is especially given as these substances e.g. induce T cells, B cells, NK cells or APCs or alter the cytokine secretion of T cells, B cells, NK cells or APCs.

It turned out, however, that administration of an antigen to-

gether with such immunostimulating substances alone does not lead to an efficient cellular immune response although such substances may have - as stated above - T cell inducing activity, are able to alter the cytokine secretion of T cells (s. Levite (1998), and Scholzen et al (1998)) or activate the immune system in a more general way.

The present invention is based on the careful selection of immunostimulating substances to be used in connection with the polycationic polymer. The polycationic polymer serves as adjuvant in the present invention. It is therefore clear that other adjuvants described in the prior art may not be regarded as immunostimulating substances in the course of the present invention. Although such additional adjuvants may be also added to the present vaccine, they cannot substitute the immunostimulating substance selected according to the present invention.

HGH is specifically preferred for the present invention as immunostimulating substance. Several isoforms are known for this protein, recombinant forms of such isoforms being specifically preferred for the present invention. Preferably, HGH is administered in a stabilized composition according to the present invention, especially with stabilizers such as glycine, tensides, mannitol or polyoxyethylene-polyoxypropylene block copolymers, and buffers, such as sodium phosphate or citrate (WO 89/09614, EP 0 211 601 A, EP 0 587 958 A).

The antigens to be used in the present compositions are not critical. Preferably proteins or peptides derived from a viral or a bacterial pathogen or from fungi or parasites are used as such antigens (including derivatized antigens like glycosylated, lipidated, glycolipidated or hydroxylated antigens. Furthermore, carbohydrates, lipids or glycolipids may be used as antigens themselves. Preferred pathogens are selected from HIV, HBV, HCV, Influenza virus, Rotavirus, Staphylococcus aureus, Chlamydia pneumoniae, Mycobacterium tuberculosis, Streptococcus pneumoniae, Bacillus anthracis, Vibrio cholerae, Plasmodium sp. (Pl. falciparum, Pl. vivax, etc.), Aspergillus sp. or Candida albicans. The derivation process may include the purification of a specific protein from the pathogen, the inactivation of the pathogen as

well as the proteolytic or chemical derivatization or stabilization of such a protein. In the same way also tumor antigens (cancer vaccines) or autoimmune antigens may be used in the pharmaceutical composition according to the present invention. With such compositions a tumor vaccination or a treatment for autoimmune diseases may be performed.

Preferably antigens are used which induce an antigen-specific type-1 like tumoral and cell mediated immune response in vivo. CD4- and CD8-epitopes are preferred, especially in connection with vaccines against pathogens and tumors.

Preferred immunostimulating substances include pituitary growth hormones or derivatives thereof, especially proteolytically or recombinantly produced derivatives which exhibit the functional properties of the growth hormone (described e.g. in the US patents 5,854,026 or 5,849,535, 5,424,199 or 5,580,723). It has been shown that such substances are also able to alter the cytokine secretion of T cells, B cells or NK cells, but they may also exert effects on APCs or B cells or NK cells. Their immunostimulating effect was connected in the literature with specific receptors on T cells (s. Levite (1998)).

Other preferred neuroactive compounds are selected from but are not restricted to growth hormones, especially human growth hormone, neurokinin A, vasoactive intestinal peptide, neuropeptide Y, substance P, Thyrotrophin (TSH), Insulin-like growth factor I (IGF-1), prolactin, lactogen, luteinizing hormone, follicle stimulating hormone, dehydroepiandrosterone (DHEA), Thymosin, Thymulin, Kentsin, melatonin, semaphorins (s. Levite (1998); Scholzen et al (1998); Aronin et al, Ann.Rev.Physiol. 48 (1986), 537-549; Berczi, Acta Paediatr Suppl. 423 (1997), 70-75; Chappel, J. Acq.Imm.Def.Synd. 20(5)(1999), 423-431; Goldman et al., Ann N.Y. Acad. Sci 419 (1983), 143-155; Spriggs, Curr.Op.Immunol. 11 (1999), 387-391; Delneste et al., J. Immunol. 163 (1999), 3071-3075). Also functional derivatives of such compounds may preferably be used in the present invention. A suitable method for providing such derivatives of naturally occurring substances is disclosed in the US 5,580,723.

It is clear to the man skilled in the art that the terms "neuro-active compounds", "hormones", "compounds having a growth hormone activity" are overlapping and many known substances fell into two or even all three groups. However, these terms are to be understood according to their meaning as generally used in the scientific literature, especially in the literature which deals with immunological research to these substance classes.

The polycationic compound to be used according to the present invention may be any polycationic compound which shows the characteristic effect according to the WO 97/30721. Preferred polycationic compounds are selected from basic polypeptides, organic polycations, basic polyaminoacids or mixtures thereof. These polyaminoacids should have a chain length of at least 4 amino acid residues (see: Tuftsin as described in Goldman et al (1983)). Especially preferred are substances like polylysine, polyarginine and polypeptides containing more than 50% of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof.

These polycationic compounds may be produced chemically or recombinantly or may be derived from natural sources. Preferred polycationic compounds derived from natural sources include HIV-REV or HIV-TAT derived cationic peptides, antennapedia peptides, chitosan (or other derivatives of chitin) and other peptides derived from these peptides or proteins by biochemical or recombinant production.

It was very surprising that with the pharmaceutical composition according to the present invention the immunostimulating effect was significantly higher than it could be expected from the addition of the effects of each single component or even the addition of the effects of the polycation with the antigen and the immunostimulating substance selected according to the present invention with the antigen. Moreover, it turned out that the effect of the selected immunostimulating substances alone is not very high when an antigen is directly applied with this substance. This is true in particular if the compounds are not repeatedly administered.

According to another aspect the present invention also relates to vaccines which comprise a composition according to the present invention.

Moreover, the present invention is also drawn to the use of the composition according to the present invention for manufacturing a vaccine.

The relative amounts of the ingredients of the present composition are highly depending on the necessities of the individual composition, e.g. the polycationic polymer to be used. In the case of poly-L-arginine and poly-L-lysine, preferred amounts of antigen/immunostimulating compound/polycation lie in the range of 1-10000 µg antigen per vaccination, 0,001 to 1000 units of immunostimulating compound per dose, especially in the case of hormones, such as HGH, and 0,1 to 1000 µg polycation.

The present compositions may be applied to a patient, e.g. a vaccination candidate, in efficient amounts e.g. by weekly, bi-weekly or monthly intervals. Patients to be treated with the present compositions may also be vaccinated repeatedly or only once. A preferred use of the present invention is the active immunisation, especially of humans or animals without protection against the specific antigen.

The route of application for the present composition is not critical, e.g. subcutaneous, intramuscular, intradermal or transdermal injection is suitable as well as oral uptake.

It is also possible to apply the present composition separately e.g. by injecting the immunostimulating substance separately from the antigen/polycation composition. The present invention is therefore also directed to a kit comprising a composition containing the antigen and the polycationic polymer as one component and a composition containing the immunostimulating or chemotactic substance as a second component.

The components may be applied at the same site or time, however, an application at different sites or at a different time or for a different time period is also possible. It is also possible to

vary the systemic or local applications of the composition or the components, respectively.

In a further aspect the present application relates to a vaccine against HCV infections comprising a polycationic polymer, an immunostimulating substance and an HCV antigen being selected from the group consisting of Core 23-44 (KFPGGGQIVGGVYLLPRRGPR), CORE 131-150 (ADLMGYIPLVGAPLGGAARA), CORE 132-140 (DLMGYIPAV), NS3 1073-1081 (CINGVCWTV), NS3 1207-1226 (SPVFTDNSSPPAVPQSFQVA), NS3 1248-1261 (GYKVLVLNPSVAAT), NS3 1585-1604 (YLVAYQATVCARAQAPPPSW), NS4 1655-1675 (VVTSTWVLVGGVLAALAAYCL), NS4 1765-1784 (MWNFISGIQYLAGLSTLPGN), NS4 1909-1929 (GEGAVQWMNRLIAFASRGNHV), NS4 1785-1804 (PAIASLMAFTAAVTSPLETTG), NS4 1787-1799 (IASLMAFTAAVTS), NS4 1805-1824 (QTLLFNILGGWVAAQLAAPG), NS4 1809-1821 (FNILGGWVAAQLA), and CD4- and CD8-epitopes with at least 6, preferably 8 to 10, amino acid long fragments of said antigens or strain variants of those antigens. Numbering of the amino acid residues relates to standard HCV nomenclature. The CD8 epitopes are especially selected from Core 31-40, Core 35-44, Core 132-140, NS3 1585-1593, NS3 1585-1594, NS4 1666-1675, NS4 1769-1777, NS4 1773-1783, NS4 1787-1795, NS4 1789-1797, NS4 1789-1798, NS4 1807-1816, NS4 1809-1817 und NS4 1811-1820.

These HCV epitopes have been shown to be surprisingly effective in vaccines containing polycationic peptides, especially polyarginine.

These carefully selected peptides are conserved in the dominant genotypes of HCV which are - especially in combination with polyarginine - highly immunogenic and therefore surprisingly effective as vaccines. Although a vast variety of peptides and epitopes have been described as potential vaccine candidates for HCV it turned out that only the above mentioned epitopes together with polycationic substances (especially polyamino acids) such as polyarginine could provide the desired results. Therefore, the present invention also relates to a vaccine comprising the selected HCV antigens together with the polyamino acid adjuvants, especially polyarginine, and optionally the immunogenic compounds according to the present invention.

Details of the present invention are described by the following examples and the figure but the invention is of course not limited thereto.

Fig.1 shows the synergistic action of poly-L-arginine and human growth hormone in the induction of antigen specific T cells.

Fig.2 shows the synergistic action of poly-L-arginine and substance P in the induction of antigen specific T cells.

Examples:

Example 1: Induction of antigen specific T cells is greatly enhanced by coinjection of combination of poly-L-arginine and human growth hormone.

<u>Mice</u>	<u>C57BL/6 (Harlan/Olac)</u>
Peptide	VYDFFVWL derived from mouse tyrosinase related protein-2. Restricted to H-2Kb (Bloom et al., 1997).
	<u>Dose: 100µg/mouse.</u>
Control peptide	SIINFEKL derived from ovalbumin. Restricted to H-2Kb (Carbone and Bevan, 1989).
Poly-L-arginine 60 (pR60)	poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA chemicals
	<u>Dose: 100µg/mouse</u>
Human Growth Hormone (HGH)	0,02 IU/injection SAIZEN, Laboratoires Serono)

Peptides were synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity.

Experimental groups (5 mice each)

- 1) TRP-2 peptide
- 2) TRP-2 peptide + HGH
- 3) TRP-2 peptide + pR 60
- 4) TRP-2 peptide + pR 60 + HGH

On day 0 mice were injected subcutaneously with a total volume of 100µl containing the above mentioned compounds. Animals were sacrificed 10 days after injection of the vaccine and mesenteric and inguinal lymph nodes harvested. Results are illustrated in Figure 1.

Lymphocytes were prepared from lymph nodes as follows: cells were passed through a 70µm sieve and washed twice with DMEM medium (GIBCO BRL) containing 2,5% fetal calf serum (FCS; SIGMA chemicals). Cells were adjusted to 10^7 cells / ml in complete medium (DMEM + 10% FCS). IFN- γ -ELISPOT assays were carried out in triplicate as described (Miyahira et al., 1995). This method is a widely used procedure allowing the quantification of antigen-specific T cells. Lymphocytes were restimulated with TRP-2 peptide or with an ovalbumin-derived peptide (SIINFEKL) with the same MHC restriction serving as negative control.

Spots representing single T cells specific for the peptide used for re-stimulation (and immunization) were counted. The number of background spots observed with cells incubated without peptide(s) was subtracted from all samples. There were no spots detected when the ovalbumine derived peptide was used. The number of spots resulting from the restimulation with the TRP-2 derived peptide are shown below for each group of mice.

Example 2: preferred antigens to be used for providing a vaccine composition according to the present invention:

1. HCV: antigens according to table 1
and the antigens disclosed in Lamonaca et al.,
Hepatology 30(4) (1999), 1088-1098.

Hepatitis C peptides

TABLE I

CD4 epitopes	Sequence	References	CD8 epitopes	Sequences	References
Core 23-44	KFPGGGQIVGGVYLLPRRGPRL	(Hoffmann et al., 1995)	Core 132-140	DLMGYIPAV	(Sarobe et al., 1998)
E2/NS1	-	-	E2/NS1 723-731	FLLLDARV	(Wentworth et al., 1996)
NS3 1248-1261	GYKVLVNLNPSVAAT	(Diepolder et al., 1997)	NS3 1073-1081	CINGVCWTV	(He et al., 1999; Rehmann et al., 1996)
Core 131-151	ADLMGYIPLVGAPLGAARA	(Hoffmann et al., 1995)			

Notes:

- Core 23-44 contains two CD8 epitopes: core 31-40 (VGGVYLLPRR) and core 35-44 (YLLPRRGPRL) (Battegay et al., 1995; Rehmann et al., 1996)
- Core 131-150 contains the CD8 epitope core 132-140 (DLMGYIPLV) (Wentworth et al., 1996)

2. HIV: antigens according to table 2

Table 2

<u>Peptide</u>	<u>Sequence</u>
Gag 77-85	SLYNTVATL
Envelope 77-85	DPNPQEVVL
POL 476-484	ILKEPVHGV

3. Epstein-Barr virus: the antigens according to table 1 of Rickinson et al Ann.Rev.Immunol. 15 (1997), 405-431.

Example 3: Co-injection of substance P and pArg

Substance P was tested as a further example for a neuroactive peptide (Marx, Science 205 (1979), 886). Substance P (RPKPQQFFGLM-NH₂; SP) was synthesized and purified according to standard procedures. Experiments were conducted as in Example 1 with the exception that spleens instead of lymph nodes were used.

Splenocytes were prepared from spleens as follows: cells were passed through a 70µm sieve and washed once with DMEM medium (GIBCO BRL). Red blood cells were lysed with "red blood cells lysis buffer" (Sigma) for 1 minute and washed twice with DMEM medium (GIBCO BRL). Cells were adjusted to 3*10⁶ cells/ml in complete medium (DEMEM+5%FCS). IFN-γ-ELISPOT was carried out as described (Miyahira et al., 1995).

Experimental groups (2mice each)

- 1) TRP-2 peptide
- 2) TRP-2 peptide + pR 60
- 3) TRP-2 peptide + pR 60 + substance P

Per injection 10 nmoles SP have been administered to mice.

The results are depicted in Fig.2. It is demonstrated with the present example that co-injection of SP and poly-L-arginine results in greatly enhanced generation of T cells in an IFN-γ-ELISPOT assay.

Claims:

1. Pharmaceutical composition comprising
 - an antigen,
 - an immunostimulating substance selected from neuroactive compounds, hormones, compounds having a growth hormone activity, and mixtures thereof, and
 - a polycationic polymer.
2. Composition according to claim 1, characterized in that the antigen is a protein derived from a viral, parasitic or bacterial pathogen.
3. Composition according to claim 1, characterized in that the antigen is a tumor antigen.
4. Composition according to claim 1, characterized in that the antigen is an autoimmune antigen.
5. Composition according to any one of the claims 1 to 4, characterized in that the polycationic compound is a basic polypeptide, an organic polycation, a basic polyaminoacid or mixtures thereof.
6. Composition according to any one of claims 1 to 5, characterized in that the polycationic compound is polylysine, polyarginine, a polypeptide containing more than 50 % of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof.
7. Composition according to any one of claims 1 to 6, characterized in that the polycationic compound is derived from the REV-protein or the TAT-protein of HIV, chitosan or other chitin derivatives.
8. Composition according to any one of claims 1 to 7, characterized in that the neuroactive compounds are selected from growth hormones, especially human growth hormone, neurokinin A, vasoactive intestinal peptide, neuropeptide Y, substance P, Thyrotrophin (TSH), Insulin-like growth factor I (IGF-1), prolactin,

lactogen, luteinizing hormone, follicle stimulating hormone, dehydroepiandrosterone (DHEA), Thymosin, Thymulin, Kentsin, melatonin, semaphorins.

9. Vaccine, comprising a composition according to any one of claims 1 to 8.

10. Use of a composition according to any one of claims 1 to 8 for manufacturing a vaccine.

11. Kit comprising

- a component containing an immunostimulating substance selected from neuroactive compounds, hormones, compounds having a growth hormone activity, and mixtures thereof, and
- a component containing a polycationic polymer and an antigen.

12. Vaccine, comprising a HCV antigen selected from the group of consisting of Core 23-44 (KFPGGGQIVGGVYLLPRRGPR), CORE 131-150 (ADLMGYIPLVGAPLGGAARA), CORE 132-140 (DLMGYIPAV), NS3 1073-1081 (CINGVCWTV), NS3 1207-1226 (SPVFTDNSSPPAVPQSFQVA), NS3 1248-1261 (GYKVLVLNPSVAAT), NS3 1585-1604 (YLVAYQATVCARAQAPPSW), NS4 1655-1675 (VVTSTWVLVGGVLAALAAAYCL), NS4 1765-1784 (MWNFISGIQY-LAGLSTLPGN), NS4 1909-1929 (GEGAVQWMNRLIAFASRGNHV), NS4 1785-1804 (PAIASLMAFTA AVTSPLTTG), NS4 1787-1799 (IASLMAFTA AVTS), NS4 1805-1824 (QTLLENILGGWVAAQLAAPG) NS4 1809-1821 (FNILGGWVAAQLA), and CD4- and CD8-epitopes with at least 6, preferably 8 to 10, amino acid long fragments of said antigens or strain variants of said antigens and a polycationic polymer.

13. Vaccine according to claim 12, wherein the fragments are selected from Core 31-40, Core 35-44, Core 132-140, NS3 1585-1593, NS3 1585-1594, NS4 1666-1675, NS4 1769-1777, NS4 1773-1783, NS4 1787-1795, NS4 1789-1797, NS4 1789-1798, NS4 1807-1816, NS4 1809-1817 und NS4 1811-1820.

14. Vaccine according to claim 12 or 13, wherein the polycationic polymer is a basic polypeptide, preferably a polyamino acid, especially polyarginine.

15. Vaccine according to any of claims 12 to 14 further com-

prising an immunostimulating substance selected from neuroactive compounds, hormones, compounds having a growth hormone activity and mixtures thereof.

1/2

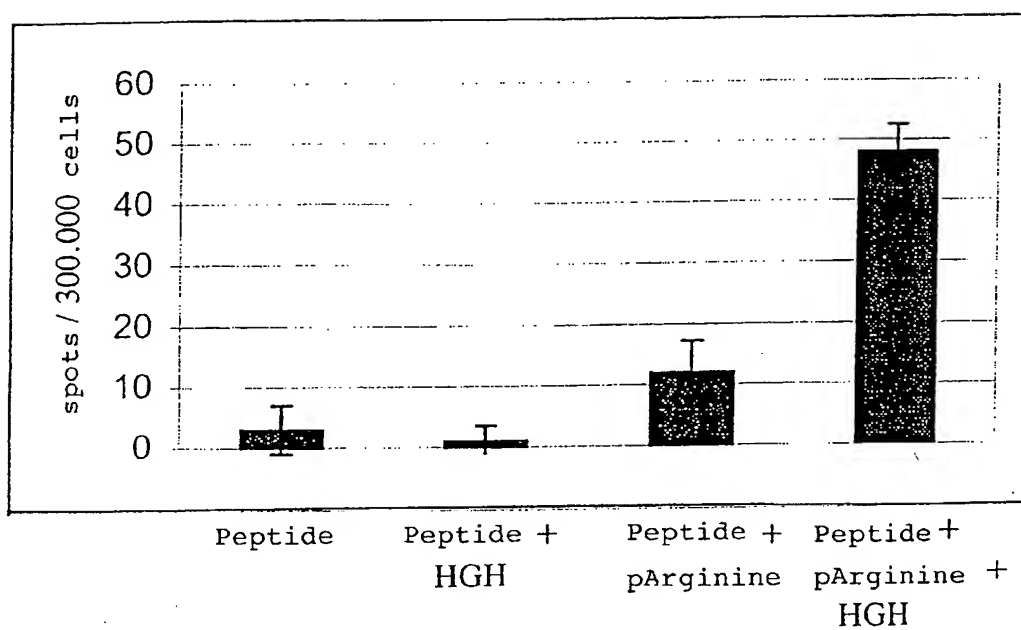
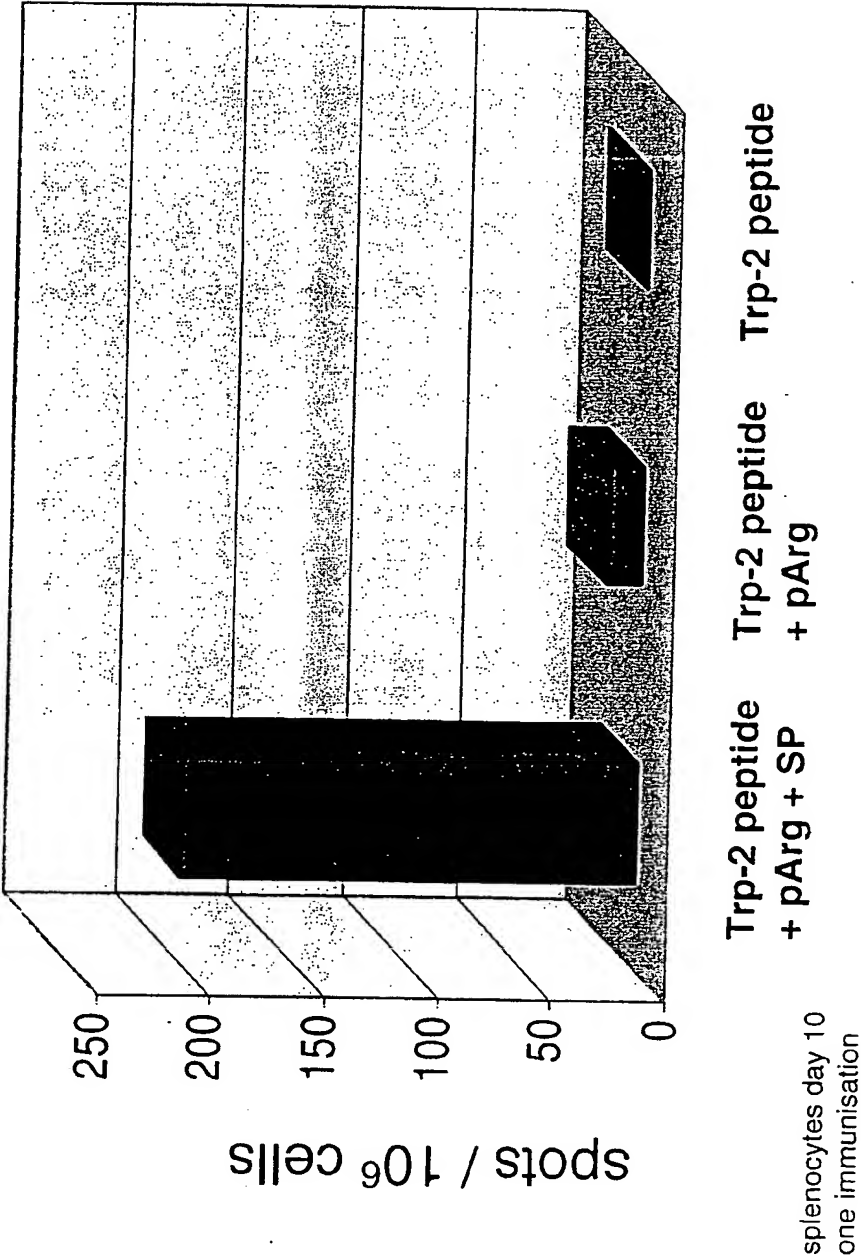


FIG. 1

FIG. 2



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☒ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.